AN EVALUATION OF BIOREGULATORS/MODULATORS AS TERRORISM AND WARFARE AGENTS

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ABSTRACT

Bioregulators or modulators are biochemical compounds, such as peptides, that occur naturally in organisms. Advances in biotechnology thus create the potential for the misuse of peptide bioregulators in offensive biological weapons programmes. They are new class of weapons that can damage nervous system, alter moods, trigger psychological changes and kill. Within neuroscience over the last twenty years has been an explosion of knowledge about the receptor systems on nerve cells that are of critical importance in receiving the chemical transmitter substances released by other nerve cells. The potential military or terrorism use of bioregulators is similar to that of toxins. Together with increased research into toxins, the bioregulators have also been studied and synthesized. This paper presents evaluation of bioregulators that can be used as terrorism delivery system or biological agents in hostile activities.

INTRODUCTION

Many biological agents have the capacity to cause disease and potentially be used to threaten civilian populations. The purpose of this paper is to provide information on bioregulators to military and health-care providers at all levels to help them make informed decisions on protecting from these agents. Bioregulators can act as neurotransmitters and modify neural response. Bioregulators are closely related to substances normally found in the body that regulates normal biological processes. Some examples of potential application of bioregulators are to cause pain, as an anesthetic and to influence blood pressure.

These substances can also modified synthetically, whereupon they may obtain new properties. It is feasible to produce some of these compounds by chemical synthesis. It is apparent that the past decade has brought an enormous increase in knowledge about the pharmacology and structural biology of receptors.

In the last ten years considerable advances have taken place in this *in vitro* synthesis of peptides and already commercial production in large quantities of various pharmaceutical peptides are freely available. Synthetic derivates or slightly modified forms of these compounds can have drastically altered toxic effects and these could be important in the development of new agents. Advances in discovery of novel bioregulators, especially bioregulators for incapacitating, understanding of their mode of operation and synthetic routes for manufacture have been very rapid in recent time. Some of these compounds may be potent enough to be many hundreds of times more effective than the traditional chemical warfare agents. Some very important characteristics of new bioregulators that would offer significant military advantages are novel sites of toxic action; rapid and specific effects; penetration of protective filters and equipment and militarily effective physical incapacitation. Peptide bioregulators are interesting regulatory molecules for many reasons. Their range of activity covers the entire living system, from mental processes (e.g. endorphins) to many aspects of health such as control of mood, consciousness, temperature control, heart rate, immune responses, sleep, or emotions, exerting regulatory effects on the body. As such, they are produced in very small quantities that are essential for the normal homeostatic functions of the body.

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They are also capable of regulating a wide range of physiological activities, such as bronchial and vascular tone and muscle contraction. This opens an unprecedented possibility to use toxic substances that could not be traced in human body. In each case a clandestine application of such substances can lead to death - "killing without a trace".

Although the use of bioregulators in military conflicts has been a concern of military communities for many years, several recent events have increased the awareness regarding the potential use of these weapons by terrorists against civilian populations. Although no known illnesses resulted from these attempts to use these agents as weapons, the exploration of the use of these agents by this and other extremist organizations caused great concern regarding the potential vulnerabilities of civilians to such weapons.

Because they must be delivered as respirable aerosols, their toxicities and ease of production limit toxins' utility as effective MCBTW. Research literature suggests that we have discovered the majority of the "most toxic" (LD_{50} < 0.0025 mg/kg) naturally occurring toxins.

Bioregulators are still considered to be less suitable for dispersal on a large scale. Nonetheless, they could be used for sabotage or in especially designed inputs, e.g., against key persons. Since bioregulators have low volatility, they are dispersed as aerosols and then taken up foremost through inhalation. The new micro encapsulation technology, which is easy to use, makes it possible to protect unstable toxins when dispersed. During recent years, discussions have started on the risk of bioregulators being used as chemical warfare agents. These types of substances do not belong to the group of toxins but are, nonetheless, grouped with them since their possible use is similar. They are closely related to substances normally found in the body and may be algogenic (causing pain), anesthetic, or influencing blood pressure. A characteristic of them is that they are active in extremely low doses and frequently have rapid effect.

One example of this group of substances is Substance P, a polypeptide (molecular weight = 1,350 D) that is active in doses of less than one microgram. Substance P causes, for example, a rapid loss of blood pressure, which may cause unconsciousness.

There are still many unknowns regarding bioregulators and their weaponization. A Mass Casualty Biological (Toxin) Weapon (MCBTW) is any toxin weapon capable of causing death or disease on a large scale, such that the military or civilian infrastructure of the state or organization being attacked is overwhelmed. A militarily significant (or terrorist) weapon is any weapon capable of affecting-directly or indirectly, physically or through psychological impact-the outcome of a military operation. Advances in the use of viral and bacterial vectors enhance the possibility for direct delivery of toxin or bioregulator to the human target or they could be used to transfer the toxin or bioregulator genes to the target. It was very hard to find in available literature all the data for all bioregulators especially for criterion: Agents known to have been developed, produced, stockpiled or used as weapons (in the tables - Weaponized).

From a public health standpoint, bioregulators which are less known, must be evaluated and prioritized in order to assure appropriate allocation of the limited funding and resources that are often found within public health systems.

Potential terrorism and warfare bioregulators were given with an expected mortality of $\geq$50% were rated higher (+++) than agents with lower expected mortalities (21-49% = ++, and <21% = +).

Bioregulators with higher rating (++) for morbidity are if clinical disease required hospitalization for treatment (including supportive care), and with lower rating (+) if outpatient treatment was possible for most cases.

Bioregulators received + to +++ for dissemination potential based on their likely methods of contamination a large area by aerosol for respiratory exposure (+++), on contamination in quantities that could affect large populations (++), and sabotage on food and water supply (+).

High level of intoxication by variety route is showed according of the kind of exposure: per oral route (+), respiratory route (++), or both (+++).

Bioregulators also were ranked based on any special public health preparedness that was required including: stockpiling of therapeutics (+), enhanced surveillance and education (+), and improved laboratory diagnostics (+).

Public fear associated with an agent and the potential mass civil disruptions that may be associated with even a few cases of disease were also considered (+ to +++).

Criteria for selection of bioregulators as terrorism agents

1. High level of morbidity: higher rating (++) if clinical disease requires hospitalization for treatment including supportive care and lower rating (+) if outpatient treatment is possible for most cases.
2. High level of mortality or incapacity: agents with an expected mortality of ≥50% were rated higher (+++), and with lower expected mortalities (21-49%=++, and <21%=+).
3. Stability in the environment after release (+)
4. Ease of production and transportation (+)
5. Likely methods for terrorism usage and high level of dissemination or contamination by aerosol for respiratory exposure (+++), contamination in quantities that could affect large populations (++), and dissemination potential for sabotage on food and water supply (+).
6. High toxicity or potency or low toxic dose: LD_{50} <0,000025 mg/kg (+++), LD_{50} from 0,000025 do 0,0025 mg/kg (++) and LD_{50} >0,0025 mg/kg (+).
7. High level of intoxication by variety route: per oral route (+), respiratory route (++), or both (+++).
8. Stockpiling of prophylactics and antidotal therapy (+)
9. Enhanced surveillance and education (+)
10. Difficult to diagnose or identify at the early stage or improved laboratory diagnostics (+).
11. Public perception: Public fear associated with an agent and the potential mass civil disruptions that may be associated with even a few cases of disease were also considered (+ to +++).

Criteria for selection bioregulators as warfare agents

1. Agents known to have been developed, produced, stockpiled or used as weapons (+).
2. High level of dissemination potential for contamination a large area by aerosol for inhalathory exposure and in military significant quantities that could affect large populations (+++), and dissemination potential for contamination of food and water supply (++).
3. High toxicity or potency or low toxic dose: LD_{50} <0,000025 mg/kg (+++), LD_{50} from 0,000025 do 0,0025 mg/kg (++) and LD_{50} >0,0025 mg/kg (+).
4. High level of morbidity: higher rating (++) if clinical disease requires hospitalization for treatment including supportive care and lower rating (+) if outpatient treatment is possible for most cases.
5. High level of intoxication by variety route: per oral route (+), respiratory route (++), or both (+++).
6. High level of mortality or incapacity: agents with an expected mortality of ≥50% were rated higher (+++), and with lower expected mortalities (21-49%=++, and <21%=+).
7. No effective prophylaxis and therapy commonly available and widely in use (+).
8. Stability in the environment (+).
9. Difficulty to diagnose/detect or identify at the early stage (+).
10. Ease of production and transportation (+).

A. ENDORPHINS

Endorphins are small-chain peptides, which activate opiate receptors, producing feeling of well-being, tolerance to pain, etc. These compounds are hundreds or even thousands of times more potent than morphine on a molar basis. Because of this potency, their concentrations in vivo are low, and it has taken recent advances in experimental neuroscience to elucidate the chemistries of these hormones. The term opioid peptides are used for the endorphins. Proopiomelanocortin - POMC (pro-ACTH-Endorphin) is a

glycosylated 31 kDa protein precursor posttranslational processing of which yields several neuroactive peptides upon specific cleavage and possibly a great number of as yet unidentified small peptides that may be pharmacologically active. Endorphins can further decompose to small fragments (oligomers) which are still active, and which pass the blood-brain barrier more readily. Their high activity and specificity make endorphins attractive compounds from a clinical view, but most are active only if injected into the blood (or the cerebrospinal fluid). This is because peptides are digested in the stomach, decomposed by proteolytic and other enzymes. Also, because of their size and structure, they have difficulty passing into the brain. Thus, despite the low oral to parenteral ratio of many morphine derivatives, they will probably not be replaced by small-chain peptides anytime soon. Dipeptidyl carboxypeptidase, enkephalinases, angiotensinases, and other enzymes accomplish enzymatic degradation of small-chain endorphins.

POMC cleavage products include a large N-terminal fragment, which yields γ-MSH (melanocyte stimulating hormone-gamma) and possibly other unidentified cleavage products, ACTH (corticotropin, 39 amino acids), Lipotropin, α-MSH (melanocyte stimulating hormone-alpha; melanotropin; acetylated and amidated ACTH 1-13), β-MSH (melanocyte stimulating hormone-beta) and β-endorphin**.** Individual products of the POMC protein act on immune cells and to be produced by them, thus establishing close links between immune cells and the nervous system. Endorphin molecules have a separate nomenclature (α, β, γ) that denotes their stereochemistry.

β-endorphin (and also α-endorphin and γ-endorphin derived from it) has been found to be produced also by macrophages and lymphocytes. β-endorphin appears to act differentially: its C-terminal moiety enhances T-cell proliferation, whereas this stimulatory effect can be prevented by peptides that possess the N-terminal enkephalin sequence. Human β-endorphin is the most potent of three stereoscopic variants, and has the same sequence as the C-terminal end of β-lipotropin.

Endorphins enhance the natural cytotoxicity of lymphocytes and macrophages towards tumor cells, stimulate human peripheral blood mononuclear cell chemotaxis and inhibit production of T-cell chemotactic factors. Opiate receptors presynaptically inhibit transmission of excitatory pathways including acetylcholine, the catecholamines, serotonin, and substance P, a neuropeptide active in pain neurons. Endorphins may also be involved in glucose regulation.

B. SUBSTANCE P (SP)

Substance P (P=powder, the designation originating from early studies using powdered extracts of equine brain and intestines). It is known also as neurokinin-1 (NK1). It is a member of a family of proteins known as tachykinins. This neuropeptide was found in the gut as well as in the brain. It is responsible for a number of excitatory effects on both central and peripheral neurons. It contracts smooth muscle, constricts bronchioles and increases capillary permeability. When released from afferent nerves, it causes a neurogenic inflammatory response, including mast cell degranulation. Substance P, a polypeptide (molecular weight = 1,350 D) which is active in doses of less than one microgramme. Substance P causes a rapid loss of blood pressure, which may cause unconsciousness.

C. ENDOTHELINS (ET) OR (EDCF-ENDOTHELIUM-DERIVED CONTRACTING FACTOR)

Endothelins are a family of closely related peptides of 21 amino acids with two disulfide bonds. The four known species are isoforms encoded by four different genes. They are called ET-1 (endothelin-1), ET-2 (endothelin-2), ET-3 (endothelin-3) and VIC (vasoactive intestinal contractor).

Endothelin is a highly potent vasoconstrictor peptide first isolated from porcine endothelial cell supernatant. Varying amounts of ET are also produced in other cell types such as smooth muscle, neuron, mesangium, melanocyte, parathyroid and amnion. Individual ET may posses separate physiological or pathophysiological roles in different target tissues. Secretion of ET is stimulated by epinephrine, angiotensin II, arginine vasopressin, transforming growth factor beta, thrombin, interleukin-1, and

hypoxia. Endothelins act to stimulate contraction of many smooth muscle tissues including blood vessels, uterus, bladder, and intestine. ET-1 is the most potent vasoconstrictor peptide yet discovered.

Numerous studies have implicated the endothelins in cardiovascular diseases such as hypertension, heart failure, and atherosclerosis. Endothelin levels are elevated in atherosclerosis, congestive cardiac failure and renal insufficiency. ET may play an important role in homeostatic hemodynamic balance. Endogenous endothelins and ET receptor subtypes are present in various endocrine organs. ET appears to act a modulator of secretion of prolactin, gonadotropins, GH and TSH. It is also may act as a neurotransmitter.

Among this family of peptides ET-1 is the most studied compound. Therapeutic potential of endothelins has generated tremendous interest in numerous laboratories around the world.

Structures of recently isolated snake venom sarafotoxins (**Sarafotoxin S6a** and **S6b**) bear striking resemblance to endothelins. They are carrying vasoconstrictor activity and potent coronary constrictor activity. They can cause heart arrest in several minutes with concentration of LD_{50} - 15 $mg.kg^{-1}$.

D. BRADYKININ (KININ-9, KALLIDIN)

Bradykinin is the final product of the kinin system and is split from a serum α-2-globulin precursor by the kallikreins and also by trypsin or plasmin. Bradykinin reduces blood pressure by dilating blood vessels. In bronchial smooth muscles and also in the intestines and the uterus bradykinin leads to muscle contraction. Bradykinin is also one the most potent known substances inducing pain. BK causes hypotension, contracts smooth muscles and increases vascular permeability. It also plays a role in pain pathways and inflammation. BK antagonists are used for treating inflammations, pain, rheumatic arthritis, osteoarthritis, pancreatitis, rhnitis, asthma and gout. Bradykinin has a powerful influence in stimulating smooth muscle contraction, inducing hypotension, increasing blood flow and permeability of capillaries.

E. VASOPRESSIN (VP)

This protein is called also antidiuretic hormone (ADH), adiuretin, vasotocin, pituitrin P and pitressin. It is a cyclic nonapeptide synthesized in the hypothalamus and stored in the posterior lobe of the pituitary from which it is released into the circulation as necessary. Functions of VP include stimulation of ATCH release, improvement of the memory and learning capacity, reduction of the pressure in the pulmonary arteries and reduction of renin and ACE activity. Vasopressin regulates osmotic pressure in body fluids via a specific vasopressor receptor (V1). It has direct antidiuretic activity in the kidney, mediated by the antidiuretic receptor V2, and promotes re-adsorption of water in the distal convoluted tubules of the kidney. It also causes vasoconstriction in peripheral small blood vessels by stimulating smooth muscle cells in the cell walls to contract.

F. ANGIOTENSINS

Angiotensin is a decapeptide originally found to be produced by kidney derived renin from an α-2 hepatic globulin. It is mainly known for its potent pharmacological activities. Angiotensin elevates blood pressure through its direct vasoconstrictor, sympathomimetic, and (through release of aldosterone) sodium-retaining activities.

Angiotensins are formed in biological fluids by the enzymatic cleavage of proteins. The species-specific enzyme renin, which can be generated by kallikrein from inactive prorenin, is responsible for the formation of angiotensin I (AT I) from globulin angiotensinogen (ATG). AT I that has no effect on the blood pressure, is split by the membrane bound angiotensin-converting enzyme (ACE) to form angiotensin II (AT II).

Angiotensin II is a very potent vasoconstrictor substance and acts directly on the adrenal gland to stimulate the release of aldosterone. The inhibition of ACE results in a double hypotensive effect because both the formation of blood pressure raising AT II as well as the degradation of the blood pressure

lowering kinin is inhibited. AT II agonists are used for treatment of shock and collapse in which a normal blood pressure could be restored as quickly as possible, while ACE inhibitors and AT II antagonists are applied as antihypertensive agents for treatment of hypertension.

G. ENKEPHALINS

These compounds comprise the basis for the body's own pain fighting mechanisms. The enkephalins are found in many areas of the body. Changes in these compounds and their metabolism have been associated with different headache disorders.

The two 5-peptide enkephalins have been identified. One terminates in a leucine, and is known as *Leu*-enkephalin; the other terminates in a methionine, and is called *Met*-enkephalin. The enkephalins are relatively weak analgesics, which activate all opioid receptors, but appear to have the highest affinity for the d receptor. Apart from nervous tissue, enkephalins have been identified in many other organ systems, including the gut, sympathetic nervous system, and adrenal glands. In the CNS, enkephalins have been found in many areas but predominantly those associated with nociception (e.g. PAG and dorsal horn). Their pre-cursor molecule is proenkephalin and they are rapidly degraded by enkephalinase. Wondering why the human brain should have receptor sites for alkaloids from the opium poppy led to the discovery of a family of natural painkillers, the endorphins (from *endogenous morphines*). These substances are oligopeptides, containing from 5 to 30 amino acids.

H. SOMATOSTATIN (SRIF)

Somatostatin, known also as somatotropin release inhibiting hormone (SIH) , is a peptide of 14 amino acids found in the hypothalamus and central and peripheral nervous system. Angiopeptin is a stable analog of somatostatin. Somatostatin (SRIF) is formed as prepro-SRIF. The main product of gene expression is pro-SRIF- (1-64), which is processed at the C-terminus to form SRIF-28 and SRIF-14. SRIF and SRIF like substance have been found in hypothalamus, central and peripheral nervous system as well as gastrointestinal tract. The main biological effect of SRIF is to inhibit the release of growth hormone, TSH, prolactin, CRH, insulin, glucagon, VIP, secretin, pancreatic polypeptide, gastrin releasing peptide, gastrin, CCK and motilin. A possible role for somatostatin in affective disorders is suggested by its low concentration in cerebrospinal fluid of patients with depression. Somatostatin in the brain might be involved in therapeutic effects of some of antidepressant drugs.

I. BOMBESIN (BN)

Bombesin is a tetrapeptide found in skins of Bombina and Bombina variegate. It distributes in the central nervous system, the gastrointestinal tract as well as the peripheral tissues. Bombesin and Bombesin-like factors show a wide spectrum of biological activities. These include regulation of the contraction of smooth muscle cells, induction of the secretion of neuropeptides and hormones. Bombesin increases the plasma levels of gastrin, CCK, glucagon, insulin, pancreatic peptide, VIP and many other gastrointestinal peptides. The C-terminal nonapeptide of bombesin has the minimum length with the maximum effect. Bombesin is used as a diagnostic aid in the gastrin stimulation test. Bombesin originally isolated from the skins of the amphibians Bombina bombina and Boombina variegata variegata, is a potent stimulant of gastric acid secretion and shown the strong biologically active in central nervous system.

J. NEUROTENISN

Neurotenisn is a 13 amino acid peptide isolated from bovine hypothalamus. It causes hypotension in the rat and its smooth muscle actions include relaxation of the rat duodenum and contraction of guinea pig ileum and rat uterus. Neurotensin may also act as a CNS neurotransmitter. Neurotensin is involved

with memory function, and that in brains of Alzheimer's disease patients there are deficits in this peptide in certain regions involved with memory function. This peptide may also be involved in the pathophysiology of Parkinson's disease and schizophrenia.

K. OXYTOCIN

The posterior pituitary has two hormones, ADH (antidiuretic hormone, vaspopressin) and oxytocin which are medically important. Both of these hormones are small peptides containg nine (9) amino acids each. They are synthesized in the hypothalamus (supraoptic nucleous for ADH and paraventricular nucleus for oxytocin). Oxytocin stimulates contraction of uterine smooth muscle. It is secreted during labor to effect delivery of the fetus. Oxytocin also stimulates contraction of smooth muscle in the mammary glands (myoepithelial cells). Oxytocin causes smooth muscle contraction in the alveoli (small chambers) and larger sinuses of the mammary glands to make readily available milk, whose production has been induced by prolactin and estrogen, to the suckling infant. Oxytocin causes milk ejection, which is necessary for adequate lactation, but not milk production. Prolactin controls milk production in conjunction with estrogen.

L.THYROTROPIN – THYROLIBERIN TSH (THYROID STIMULATING HORMONE)

A glycoprotein hormone consisting of two protein chains, one of which is identical with a subunit of Luteinizing hormone. Thyrotropin is produced in the anterior pituitary in response to thyrotropin releasing hormone (thyroliberin; thyrotropic hormone releasing factor or TRF).

Thyrotropin stimulates the thyroid gland to secrete thyroid hormones such as thyroxin and triiodothyronin. These two hormones inhibit the secretion of TRF and thyrotropin. Thyrotropin stimulates secretion of prolactin and acts as a neurotransmitter in the central nervous system. Apart from its well-known physiological role thyrotropin appears to be involved in the modulation of immune responses within the neuroimmune network. Thyrotropin enhances proliferation of lymphocytes stimulated by suboptimal concentrations of IL2 and enhances IL2-induced NK-cell activity. TSH also enhances production of superoxide anions by stimulated macrophages.

M. HRF (HISTAMINE-RELEASING FACTORS)

This is a general term used for factors that induce the release of histamines from basophils and mast cells when stimulated with antigens or mitogens.

HRIF (Histamine release inhibitory factor)

This poorly characterized factor is a specific antagonist of histamine-releasing factors. It is produced by peripheral blood mononuclear cells (B-cells, T-cells, monocytes) upon stimulation with histamine or mitogens such as Con A. It inhibits HRF-induced histamine release from basophils and mast cells. One particular factor with HRIF activity is IL8 .

CTAP-3 Beta-Thromboglobulin (Beta-TG)

CTAP-3 (connective tissue activating protein-3) or beta-thromboglobulin is a protein of 8.85 kDa. Beta-thromboglobulin is stored in the Alpha-granules of platelets and released in large amounts after platelet activation. Beta-thromboglobulin is a strong chemoattractant for fibroblasts and is weakly chemotactic for neutrophils. It stimulates mitogenesis, extracellular matrix synthesis, glucose metabolism, and plasminogen activator synthesis in human fibroblast cultures.

Beta-thromboglobulin, its precursor, and its cleavage products influence the functional activities of neutrophilic granulocytes. Beta-thromboglobulin affects the maturation of human megakaryocytes and thus could play a role in the physiological regulation of platelet production by megakaryocytes.

N. AND O. NEUROKININ A (NKA) (SUBSTANCE K), NEUROKININ B (NKB) (NEUROMEDIN K)

Neurokinins are found centrally in the spinal cord and in the sensorial nuclei of the brain stem and peripherally in the ends of the sensorial fibers. Neurokinins include Substance P (SP), neurokinin A (NKA), and neurokinin B (NKB). Similar compounds, which occur in cold-blooded animals, are called tachykinins. So far, three different receptors have been found for the neurokinins: NK1 for SP, NK2 for NKA, NK3 for NKB. Neurokinins play various roles in the regulation of cardiovascular system, pain pathway and inflammatory reaction. Neurokinin A and B belong to the tachykinin family. They are a more potent bronchio-constrictor than substance P and may regulate neutrophil recruitment in the lower respiratory tract. They arise from larger precursor molecules and exhibit functions such as vasodilatation, hypotension, extravascular smooth muscle contraction, salivation and increase of capillary permiability

P. NEUROPEPTIDE Y (NPY)

Neuropeptide Y (NPY) is present in the brain and in the peripheral nervous system along with other neurotransmitters. It has structural homology with pancreatic polypeptide (PP) and peptide YY (PYY). Its functions may include neurotransmission, neuromodulation, vasoconstriction, regulation of blood pressure, and appetite.

Our opinion is that if some bioregulator satisfies the bulk of the criteria, it should be recommended for inclusion in the list. As the list of bioregulators will be hard to define generally and for purposes of the future negotiations of the States Parties of BTWC, this paper proposes two tables of enlisted bioregulators with important criteria on the basis of which a decision can be made to include in or exclude from a list of the molecular agents (bioregulators). Rankings of potential bioregulators according to important criteria are shown in:

Table 1. Bioregulator assessment according to criteria for selecting bioregulators as warfare agents, and

Table 2. Bioregulator assessment according to criteria for selecting bioregulators as terrorism agents.

CONCLUSIONS

All of that shows that it is very hard to make a final decision on criteria and the final list of the molecular agents (bioregulators) for the needs of future Protocol to the BTWC based on these criteria. Because of all of that, this paper proposes that list and criteria for bioregulators be well studied and that an opinion by scientists and experts be obtained, because the list should be scientifically based. Although many bioregulators can be used to cause illness, they can truly threaten civilian populations on a large scale. If released upon a civilian population, these agents would pose the most significant challenge for public health and medical responses.

Although many biological agents such as bioregulators can be used to cause illness, there are only a few that can truly threaten civilian populations on a large scale. If released upon a civilian population, these agents would pose the most significant challenge for public health and medical responses. The above criteria for ranking potential bioregulators and listing of them of greatest public health concern could be used for determination of priority biological threat agents for national public health preparedness efforts for bioterrorism. Having a defined method for evaluating biological threat agents allows for a more objective evaluation of newly emerging potential threat agents, as well as continued re-evaluation of established threat agents. Using this prioritization method can help focus public health activities related to bioterrorism detection and response and assist with the allocation of limited public health resources.

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Table 1. Bioregulator assessment according to criteria for selecting bioregulators as warfare agents

Bioregulator	**(1) Weapo-nized** (+)	**(2) High level of dissemi-nation** (+++)	**(3) High tox-icity** (+++)	**(4) High morbi-dity** (++)	**(5) Intoxication by variety of route: per oral route, respiratory route, or both** (+++)	**(6) High level of incapacity/ mortality** (+++)	**(7) No effective prophylaxis and therapy** (+)	**(8) Stability in the environ-ment** (+)	**(9) Difficulty of detection/ identifi-cation** (+)	**(10) Ease of produc-tion** (+)	**Total** (19)
1. Endorphins (α, β, and δ-Endorphin)	+	+++	+++	++	+++	+++	+	+	+	+	**19**
2. Substance P (SP) (Neurokinin)	+	+++	+++	++	+++	+++	+	+	+	+	**19**
3. Endothelins (ET-1, ET-2, ET-3) or Sarafotoxins (S6a, S6b)	+	+++	+++	++	+++	+++	+	+	+	+	**19**
4. Bradykinin (Kinin-9, Kallidin)	+	+++	+++	++	+++	+++	+	+	+	+	**19**
5. Vasopressin (VP)	+	+++	+++	++	+++	++	+	+	+	+	**18**
6. Angiotensins (I, II, III)	+	+++	+++	++	+++	++	+	+	+	+	**18**
7. Enkephalins (*Leu*- and *Met*-enkephalin)	+	+++	+++	++	+++	++	+	+	+	+	**18**
8. Somatostatin (SS, SRIF)	+	+++	++	++	+++	++	+	+	+	+	**17**
9. Bombesin (BN)	+	+++	++	++	+++	++	+	+	+	+	**17**
10. Neurotensin	+	+++	++	++	+++	++	+	+	+	+	**17**
11. Oxytocin	+	+++	++	++	+++	++	+	+	+	+	**17**
12. Thyroliberins (Thyrotropin)	+	+++	++	++	+++	++	+	+	+	+	**17**
13. Histamine releasing factors (HRF): **- Histamine release inhibiting factor (HRIF)** **- CTAP-3 Beta-Thromboglobulin (Beta-TG)** **- Neutrophil-activacting factor (NAF)** **- Stem cell factor (SCF)**	+	+++	++	++	+++	++	+	+	+	+	**17**
14. Neurokinin A (NKA)/Substance K (SK)	-	+++	++	++	+++	++	+	-	+	+	**15**
15. Neurokinin B (NKB)/Neuromedin K	-	+++	++	++	+++	++	+	-	+	+	**15**
16. Neuropeptide Y (NPY)	-	+++	++	++	++	++	+	-	+	+	**15**

Table 2. Bioregulator assessment according to criteria for selecting bioregulators as terrorism agents

Bioregulator	(1) High morbi-dity (++)	(2) High level of mortality/ incapacity (+++)	(3) Stability in the environ-ment (+)	(4) Ease of produc-tion (+)	(5) High level of dissemi-nation (+++)	(6) High tox-icity (+++)	(7) High level of intoxi-cation (+++)	(8) No effective prophylaxis and antidotal therapy (+)	(9) Enhanced surveillance and education (+)	(10) Difficulty of detection/ identifi-cation (+)	(11) Public percep-tion (+++)	Total (22)
1. Endorphins (α, β, and δ-Endorphin)	++	+++	+	+	+++	+++	+++	+	+	+	+++	**22**
2. Substance P (SP) (Neurokinin)	++	+++	+	+	+++	+++	+++	+	+	+	+++	**22**
3. Endothelins (ET-1, ET-2, ET-3) or Sarafotoxins (S6a, S6b)	++	+++	+	+	+++	+++	+++	+	+	+	+++	**22**
4. Bradykinin (Kinin-9, Kallidin)	++	+++	+	+	+++	+++	+++	+	+	+	+++	**22**
5. Vasopressin (VP)	++	+++	+	+	+++	+++	+++	+	+	+	+++	**22**
6. Angiotensins (I, II, III)	++	+++	+	+	+++	+++	+++	+	+	+	+++	**22**
7. Enkephalins (*Leu*- and *Met*-enkephalin)	++	+++	+	+	+++	+++	+++	+	+	+	+++	**22**
8. Somatostatin (SS, SRIF)	++	++	+	+	+++	+++	+++	+	+	+	+++	**21**
9. Bombesin (BN)	++	++	+	+	+++	+++	+++	+	+	+	+++	**21**
10. Neurotensin	++	++	+	+	+++	+++	+++	-	+	+	+++	**21**
11. Oxytocin	++	++	+	+	+++	+++	+++	+	+	+	+++	**21**
12. Thyroliberins (Thyrotropin)	++	++	+	+	+++	+++	+++	+	+	+	+++	**21**
13. Histamine releasing factors (HRF): **- Histamine release inhibiting factor (HRIF)** **- CTAP-3 Beta-Thromboglobulin (Beta-TG)** **- Neutrophil-activacting factor (NAF)** **- Stem cell factor (SCF)**	++	++	+	+	+++	+++	+++	+	+	+	+++	**21**
14. Neurokinin A (NKA)/Substance K (SK)	++	++	-	+	+++	+++	+++	+	+	+	+++	**20**
15. Neurokinin B (NKB)/Neuromedin K	++	++	-	+	+++	+++	+++	+	+	+	+++	**20**
16. Neuropeptide Y (NPY)	++	++	-	+	+++	+++	+++	+	+	+	+++	**20**